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Review

Linoleic Acid Derivative DCP-LA Prevents Tau Phosphorylation by Targeting GSK-3β

Tomoyuki Nishizaki[1,*]

[1] Innovative Bioinformation Research Organization, 2-3-14 Katsuragi, Kita-ku, Kobe 6751-1223, Japan; tnishizaki@bioresorganization.com (T.N.)

* Correspondence: tnishizaki@bioresorganization.com (T.N.)

Abstract: Abnormal Tau phosphorylation and aggregation into neuronal paired helical filaments and neurofibrillary tangles cause tauopathies, a class of neurodegenerative diseases, that include Alzheimer's disease, frontotemporal dementia and parkinsonism linked to chromosome 17, progressive supranuclear palsy, Pick's disease, and corticobasal degeneration. Glycogen synthase kinase-3β (GSK-3β) is the most critical kinase to phosphorylate Tau. We have developed the linoleic acid derivative 8-[2-(2-pentyl-cyclopropylmethyl)-cyclopropyl]-octanoic acid (DCP-LA), with cyclopropane rings instead of *cis*-double bonds, as an anti-dementia drug. DCP-LA serves as a selective activator of PKCε and a potent inhibitor of protein tyrosine phosphatase 1B (PTP1B). DCP-LA prevents Tau phosphorylation due to PKCε-mediated direct inactivation of GSK-3β, to PKCε/Akt-mediated inactivation of GSK-3β, and to receptor tyrosine kinase-mediated inactivation of GSK-3β in association with PTP1B inhibition. DCP-LA targeting GSK-3β, thus, could become a valid drug for treatment of tauopathies.

Keywords: DCP-LA; PKCε; protein tyrosine phosphatase 1B; Akt; GSK-3β; tauopathy

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1. Introduction

Alzheimer's disease (AD), a neurodegenerative disease, is characterized by extensive deposition of amyloid β (Aβ) called amyloid plaque and formation of neurofibrillary tangles (NFTs). Aβ has been long thought to be the most causative factor in AD, and therefore, a variety of AD drugs targeting Aβ have been developed. No beneficial result, however, has been obtained even though Aβ was cleaned up from the brain. A current challenge, therefore, has focused upon Tau protein.

Tau, a microtubule-associated protein, is abundantly expressed in neurons of the central nervous system. Tau is upregulated during neuronal development, to promote generation of cell processes and establish cell polarity [1]. Microtubules are the tracks for motor proteins bearing intracellular cargo transport [2,3]. Tau polymerizes and stabilizes microtubules by interacting with tubulin, and modulates microtubule dynamics including axonal transport [4-7].

When hyperphosphorylated, Tau detaches from the microtubules and forms insoluble fibrils, referred to as paired helical filaments (PHFs), followed by NFTs comprising aggregation of PHFs [8,9]. Aggregation of hyperphosphorylated Tau is responsible for tauopathies, a class of neurodegenerative diseases, that include not only AD but frontotemporal dementia and parkinsonism linked to chromosome 17, progressive supranuclear palsy, Pick's disease, and corticobasal degeneration [10].

Tau is phosphorylated by serine/threonine protein kinases such as GSK-3β, cyclin-dependent kinase 5 (Cdk5)/p25, extracellular signal-regulated kinase 2 (ERK2), p70 ribosomal protein S6 kinase (p70-S6K), microtubule affinity-regulating kinase (MARK), SAD kinase (SADK), protein kinase A (PKA), calcium/calmodulin-dependent protein kinase II (CaMKII) or tyrosine kinases such as Fyn and c-Abl [11-15]. Of the protein kinases GSK-3β plays a central role in Tau phosphorylation relevant to tauopathies. The present review shows that the linoleic acid derivative DCP-LA is capable of preventing Tau phosphorylation by targeting GSK-3β.

2. Tau phosphorylation in the AD brain

Tau from the AD brain contains eleven Ser/Thr-Pro sites, that are phosphorylated by proline-directed kinases, and nine Ser/Thr-X sites, that are phosphorylated by non-proline-directed kinases. GSK-3β, Cdk5/p25, ERK2, and p70-S6K belong to proline-directed kinases, which phosphorylate Tau at Thr181, Ser202/T205, Thr212/S214,

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Thr231/Ser235, and Ser396/Ser404 on Ser-Pro or Thr-Pro motifs in the regions flanking the repeat domains [11-13]. MARK, SADK, PKA, and CaMKII belong to non-proline-directed kinases, which phosphorylate Tau at Ser262, Ser 320, Ser324, and Ser356 on KXGS motifs in the repeat domains (R1-R4) [12,14,15]. The non-receptor tyrosine kinases (non-RTKs) Fyn and c-Abl phosphorylate Tau at Tyr-18 and Tyr-394 [12].

GSK-3β is enriched in the brain and preferentially expressed in the hippocampus. GSK-3β acts as the main executioner of Tau phosphorylation in PHFs [16,17]. Intriguingly, GSK-3 accelerates the rate of Tau phosphorylation several-fold, if Tau is pre-phosphorylated by priming kinases such as non-proline-directed kinases [18-20]. Of Tau phosphorylation sites, Ser396 phosphorylation is a key step in the PHF formation [21]. Once a priming kinase phosphorylates Tau at Ser404, GSK-3β phosphorylates Tau at Ser400, followed by sequential phosphorylation of Ser396 [21]. GSK-3β, alternatively, phosphorylates Tau at Ser202 directly, but Thr231 phosphorylation requires for Ser235 pre-phosphorylation [21].

3. Interaction between Aβ and GSK-3β

GSK-3β is originally in the active form. GSK-3β is inactivated by being phosphorylated at Ser9 and activated by being phosphorylated at Tyr216 [22].

Aβ activates the non-RTK Fyn, that in turn, activates GSK-3β by phosphorylating at Tyr216, leading to Tau phosphorylation and accumulation in neurons [23,24]. Aβ, alternatively, inhibits/inactivates phosphoinositide 3-kinase (PI3K), thereby suppressing GSK-3β-Ser9 phosphorylation, to activate GSK-3β [25]. Chronic exposure of Aβ downregulates Akt phosphorylation, to activate GSK-3β and increase Tau phosphorylation [26]. Soluble Aβ oligomers inhibit insulin signaling relevant to Akt activation, to activate GSK-3β and increase Tau phosphorylation [27]. Intracellular $A\beta_{1-42}$ promotes Tau phosphorylation and induces neuronal loss [28]. GSK-3β exacerbates Aβ-induced neurotoxicity and cell death [29].

Amyloid precursor protein (APP) intracellular domain (AICD), that is produced from □-secretase-mediated APP cleavage, activates GSK-3β [30] or enters the nucleus and activates gene transcription, increasing the GSK-3β mRNA and protein [31]. C-terminal fragments of APP stimulate GSK-3β activation, to increase Tau phosphorylation and induce apoptosis [32].

Aging, inflammation, and stress also activate GSK-3β, causing an initial Tau phosphorylation, responsible for mild cognitive impairment (MCI), a preliminary group of AD. Aβ further activates GSK-3β and accelerates Tau phosphorylation, leading to progression into

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AD from MCI [33,34].

4. Regulation of GSK-3β activity

The serine/threonine protein kinases such as PKCε [35], Akt [35], PKA [36], integrin-linked kinase (ILK) [37], CaMKII [38], p90 ribosomal protein S6 kinase (p90RSK) [39], and cGMP-dependent protein kinase 1 (PrkG1) [40] inactivate GSK-3β by directly phosphorylating at Ser9 (Figure 1). Conversely, inhibition of those kinases allows relative activation of GSK-3β.

Pyk2 binds to and activates SH2 and SH3 domain-containing proteins like Src kinases. Pyk2 and Fyn activate GSK-3β by phosphorylating at Tyr216 directly [23,41] (Figure 1).

Akt1 is activated by being phosphorylated at Thr308 and Ser473 through the major pathway along a RTK/insulin receptor substrate 1 (IRS-1)/PI3K/3-phosphoinositide-dependent protein kinase 1 (PDK1)/Akt axis, to phosphorylate GSK-3β at Ser9 and inactivate GSK-3β [35] (Figure 2). Accordingly, activation of RTK induces inactivation of GSK-3β; conversely, inhibition of RTK induces activation of GSK-3β. For example, insulin or insulin-like growth-factor 1 (IGF1) binds to and activates the RTK insulin receptor involving GSK-3β inactivation, which occurs still in the brain.

Lines of evidence have pointed to other pathways for regulation of GSK-3β activity. AMP-activated protein kinase (AMPK) phosphorylates and inactivates GSK-3β [42], while AMPK by itself phosphorylates Tau at Ser262 [43]. $A\beta_{1\text{-}42}$ upregulates expression of adenylate kinase-1 (AK1), that inhibits AMPK, causing GSK-3β activation [43]. AMPK□ is activated due to inhibition of protein phosphatase 2A (PP2A) through a sphingosine-1-phosphate receptor 1 (S1PR1) linked to G_i protein [44], thereby inactivating GSK-3β.

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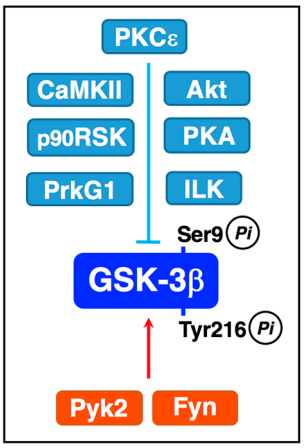

Figure 1. Protein kinases bearing phosphorylation of GSK-3β. PKCε, Akt, PKA, ILK, CaMKII, p90RSK, and PrkG1 phosphorylate GSK-3β at Ser9 and inactivate GSK-3β. Pyk2 and Fyn phosphorylate GSK-3β at Tyr216 and activate GSK-3β.

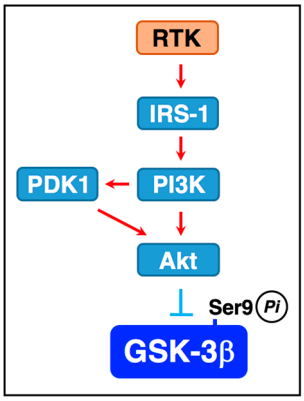

Figure 2. RTK-mediated GSK-3β inactivation. Akt is activated through a pathway along a RTK/IRS-1/PI3K/PDK1/Akt axis and inactivates GSK-3β by phosphorylating at Ser9.

5. Linoleic acid derivative DCP- LA

PKC is classified into the conventional PKC isozymes α, βI, βII, and γ, the novel PKC isozymes δ, ε, η, and θ, the atypical PKC isozymes ι/λ and ζ, and the PKC-like isozymes μ and ν. All the PKCs have the phosphatidylserine (PS) binding site and are activated by diacylglycerol (DG) [45]. Of the PKC isozymes *cis*-unsaturated free fatty acids (uFFAs) such as arachidonic, linoleic, linolenic, oleic, and docosahexaenoic acid directly interact with the novel PKC isozymes [45]. uFFAs are very unstable, and therefore, we have originally synthesized the linoleic acid derivative 8-[2-(2-pentyl-cyclopropylmethyl)-cyclopropyl]-octanoic acid (DCP-LA),

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with cyclopropane rings instead of *cis*-double bonds, that exhibits stable bioactivities [46].

The primary site of action of DCP-LA is PKC*ε*. DCP-LA activates PKC*ε* selectively and directly still in the absence of DG and calcium [47]. DCP-LA binds to the PS binding/associating sites Arg50 and Ile89 in the C2-like domain of PKC*ε* at the carboxyl-terminal end and the cyclopropane rings, respectively, which are distinct from the phorbol 12-myristate 13-acetate (PMA) binding site in the C1 domain [48] (Figure 3).

DCP-LA, on the other hand, acts as a potent inhibitor of protein tyrosine phosphatase 1B (PTP1B) through its direct interaction [49].

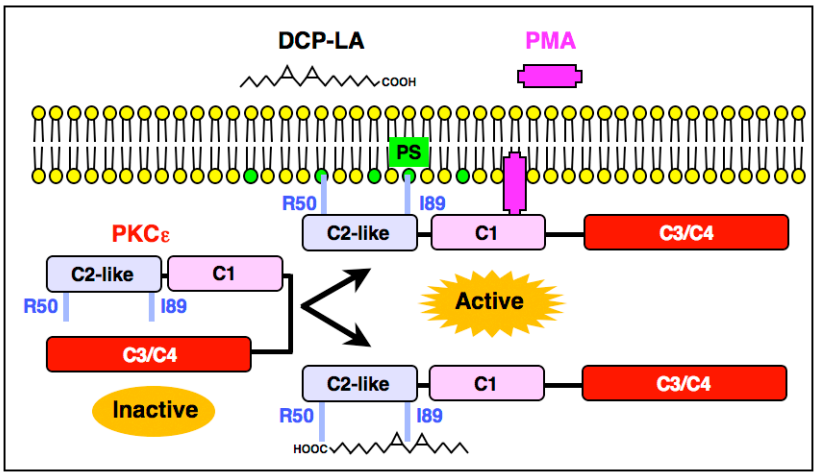

Figure 3. A schematic diagram for DCP-LA- and PMA-induced PKCε activation. Inactive form of PKCε binds to PS in the inner leaflet of the lipid bilayer at Arg50 and Ile89, to make PKCε an open frame and activate PKCε partially, allowing PMA to bind to the C1 domain and activate PKCε fully. In contrast, DCP-LA activates PKCε in the cytosol by directly interacting with Arg50 and Ile89 in the C2-like domain, regardless of PS.

6. DCP-LA inactivates GSK-3β by cooperation of PKC*ε* activation and PTP1B inhibition

PKC*ε*, activated by DCP-LA, inactivates GSK-3β by directly phosphorylating at Ser9 (Figure 4) [35]. Activated PKC*ε*, alternatively, activates Akt by directly phosphorylating at the serine residue, followed by inactivation of GSK-3β [35] (Figure 4).

When activated, RTK phosphorylates its own receptor at Tyr1185 and activates IRS-1 by phosphorylating at Tyr1222. Activated IRS-1 recruits and activates PI3K, which produces phosphatidylinositol 3,4,5-triphosphate (PIP_3) by phosphorylating phosphatidylinositol 4,5-bisphosphate (PIP_2). PIP_3 binds to and activates PDK1. PI3K and/or PDK1 activate Akt by

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phosphorylating at the serine and threonine residues. RTK and IRS-1 are inactivated through PTP1B-mediated tyrosine dephosphorylation. DCP-LA-induced PTP1B inhibition, therefore, represses inactivation of RTK and IRS-1, allowing relative Akt activation through a RTK/IRS-1/PI3K/PDK1/Akt pathway, to phosphorylate and inactivate GSK-3β [35] (Figure 4).

PKCε activation or PTP1B inhibition is capable of inactivating GSK-3β each independently. In experiments using PC-12 cells, PKCε overexpression and PTP1B deficiency activate Akt and inactivate GSK-3β synergistically [35]. This indicates that DCP-LA enables more efficient inactivation of GSK-3β by cooperation of PKCε activation and PTP1B inhibition [35] (Figure 4).

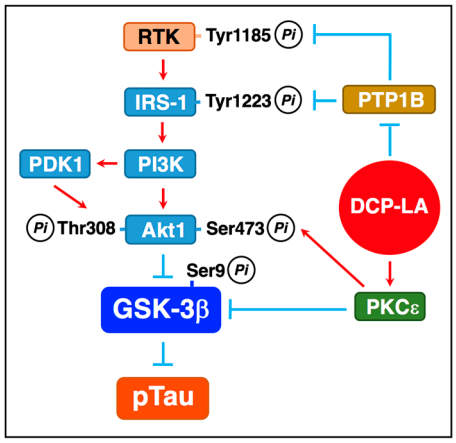

Figure 4. DCP-LA-induced suppression of Tau phosphorylation. PKCε, activated by DCP-LA, inactivates GSK-3β by phosphorylating Ser9 directly or through a PKCε/Akt pathway, to restrain Tau phosphorylation (pTau). DCP-LA-induced PTP1B inhibition, alternatively, activates Akt through a RTK/IRS-1/PI3K/PDK1/Akt pathway by repressing tyrosine dephosphorylation of RTK and IRS-1, followed by GSK-3β-Ser9 phosphorylation and inactivation of GSK-3β, to restrain pTau.

7. DCP-LA prevents Tau phosphorylation by targeting GSK-3β

$A\beta_{1\text{-}42}$ activates GSK-3β by reducing GSK-3β-Ser9 phosphorylation, leading to an enhancement in the Tau phosphorylation at Ser202/Thr205 and Ser396, and the $A\beta_{1\text{-}42}$-induced Tau phosphorylation is neutralized by DCP-LA [35]. This indicates that DCP-LA prevents Tau phosphorylation by inactivating GSK-3β with PKCε activation and PTP1B inhibition.

5xFAD mouse, as an animal model of AD, is APP/presenilin 1 (PS1) double transgenic mice that coexpress five familial forms of AD mutations such as the Swedish/London/Florida

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mutations and the M146L/L286V mutations [50]. The $A\beta_{1-42}$ levels in the 5xFAD mouse brain rise in an age-dependent manner [50]. The GSK-3β activity is enhanced in parallel with $A\beta_{1-42}$ rise and Tau-Ser396 phosphorylation, responsible for PHF formation, is accelerated in the hippocampus of 5xFAD mice [51]. DCP-LA suppresses the GSK-3β activation and Tau-Ser396 phosphorylation in the hippocampus of 5xFAD mice [35]. DCP-LA, thus, has the potential to restrain Tau-Ser396 hyperphosphorylation efficiently by activating PKCε and inhibiting PTP1B simultaneously.

8. DCP-LA ameliorates cognitive disorders by facilitating hippocampal synaptic transmission

Tau hyperphosphorylation causes tauopathies. Cognitive decline in association with tauopathies including AD, however, could not be improved only by suppression of Tau phosphorylation.

DCP-LA promotes vesicular transport of α7 ACh receptor towards the cell surface in a PKCε-dependent manner [52,53]. DCP-LA-induced increase in α7 ACh receptor on the plasma membrane at presynaptic terminals stimulates presynaptic glutamate release [52,54]. DCP-LA, alternatively, promotes exocytosis of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, a major postsynaptic excitatory receptor, through CaMKII activation due to PP-1 inhibition [55]. Those effects of DCP-LA on α7 ACh receptor and AMPA receptor induce a long-lasting facilitation of hippocampal synaptic transmission, to enhance cognitive functions [46,56].

Indeed, DCP-LA ameliorates spatial learning and memory decline in 5xFAD mice [35]. Moreover, DCP-LA improves $A\beta_{1-40}$- and mutant Aβ-induced spatial learning deficits in rats [57,58], scopolamine-induced spatial learning and memory impairment in rats [57], spatial learning and memory deterioration in senescence accelerated mice [59,60]. Overall, DCP-LA could improve cognitive disorders in a variety of dementias.

Interestingly, DCP-LA protects neurons from oxidative stress-induced apoptosis by inhibiting caspase-3/-9 activation [61]. This indicates that DCP-LA could also prevent progression of neuronal loss in the AD brain.

9. Conclusions

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DCP-LA restrains Tau phosphorylation efficiently due to PKCε-mediated direct inactivation of GSK-3β, to PKCε/Akt-mediated inactivation of GSK-3β, and to RTK/IRS-1/PI3K/PDK1/Akt-mediated inactivation of GSK-3β in association with PTP1B inhibition. In addition, DCP-LA induces a long-lasting facilitation of hippocampal synaptic transmission, to enhance cognitive functions. Taken together, DCP-LA could not only prevent Tau phosphorylation but improve cognitive impairments associated with tauopathies. Consequently, DCP-LA could be developed as a promising drug for prevention and therapy of tauopathies.

Conflict of Interest: The author declares no conflict of interest.

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